

Claims

1. A method for evaluating the cardiovascular effect of a candidate agent, comprising the steps of:
 - 5 (a) contacting an estrogen receptor-enhanced vascular endothelial cell with a candidate agent; and
 - (b) determining whether the candidate agent modulates expression of at least one estrogen-regulated marker, wherein the expression of the marker is previously
 - 10 determined to be induced by estrogen in estrogen receptor-enhanced vascular endothelial cells, and therefrom determining whether the candidate agent has a cardiovascular effect.
- 15 2. A method according to claim 1, wherein the cell is immortalized.
3. A method according to claim 1, wherein step (b) comprises the steps of:
 - 20 (i) detecting a level of an estrogen-regulated marker; and
 - (ii) comparing the detected level of the estrogen-regulated marker with a level of the estrogen-regulated marker in the absence of the candidate agent.
- 25 4. A method according to claim 1, wherein step (b) comprises the steps of:
 - (i) detecting a level of mRNA encoding an estrogen-regulated marker; and
 - (ii) comparing the detected level of mRNA encoding the
 - 30 estrogen-regulated marker with a level of mRNA encoding the estrogen-regulated marker in the absence of estrogen-related compound.
5. A method according to claim 4, wherein the level of
- 35 mRNA is detected using an Rnase protection assay.

6. A method according to claim 4, wherein the level of mRNA is detected by:

preparing cDNA from mRNA present within the cell; and
amplifying mRNA encoding the estrogen-regulated marker
5 via polymerase chain reaction.

7. A method according to claim 6, wherein the amplification is performed using real-time PCR analysis.

10 8. A method according to claim 1, wherein the cell is transfected with a polynucleotide comprising a promoter for an estrogen-regulated marker, operably linked to a reporter gene.

15 9. A method according to claim 8, wherein step (b) comprises the steps of:
(i) detecting a level of reporter protein activity; and
(ii) comparing the level of reporter protein activity detected with a level of reporter protein in the absence of
20 candidate agent.

10. A method according to claim 8, wherein step (b) comprises the steps of:
(i) detecting a level of reporter mRNA; and
25 (ii) comparing the level of reporter mRNA detected with a level of reporter mRNA in the absence of estrogen-related compound.

11. A method according to claim 1, wherein the ability of
30 the candidate agent to modulate expression of at least two estrogen-regulated markers is determined.

12. A method according to claim 1, wherein the estrogen-regulated marker is selected from the group consisting of
35 SEQ ID NOs: 1-19

13. A method for identifying a selective estrogen receptor modulator (SERM), comprising the steps of:

(a) contacting at least two different cells that express an estrogen-regulated marker expression with a candidate SERM;

5 (b) determining levels of expression of at least one estrogen-regulated marker in the cells; and

(c) comparing the levels of expression with levels of expression of the estrogen-regulated marker(s) in cells following contact with estrogen, and therefrom determining
10 whether the candidate SERM is a selective estrogen receptor modulator.

14. A method according to claim 13, wherein the estrogen-regulated marker is selected from the group consisting of
15 SEQ ID NOs: 1-19

15. A method according to claim 13, wherein levels of at least two estrogen-regulated markers are determined.

20 16. A method according to claim 13, wherein each of the cells is independently selected from the group consisting of bone, urogenital, cardiovascular, breast, ovarian, endometrial, and central nervous system cells.

25 17. A method according to claim 13, further comprising the steps of:

(d) contacting breast cells that express an estrogen-regulated marker expression with estrogen and a candidate SERM;

30 (e) determining levels of expression of at least one estrogen-regulated marker in the breast cells; and

(f) comparing the levels of expression with levels of expression of the estrogen-regulated marker(s) in the breast cells following contact with estrogen, in the absence of
35 candidate SERM.

18. A method for inhibiting the development of a cardiovascular disease in a patient; comprising administering to a patient an agent identified according to the method of claim 1 or a SERM identified according to
5 claim 13.

19. A method for inhibiting the development of breast cancer in a patient; comprising administering to a patient a SERM identified according to the method of claim 17, wherein
10 the SERM inhibits induction of estrogen-regulated marker expression by estrogen in breast cells.

20. A method for inhibiting the development of osteoporosis in a patient; comprising administering to a patient a SERM
15 identified according to claim 13.

21. An isolated or recombinant nucleic acid comprising SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; or SEQ ID NO: 19.
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22. An isolated or recombinant nucleic acid that hybridizes under stringent conditions to the nucleic acid of SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ
30 ID NO: 18; or SEQ ID NO: 19.

23. An isolated or recombinant nucleic acid that is at least 75% identical to at least 50 consecutive nucleic acids of SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID
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NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; or SEQ ID NO: 19.

24. The nucleic acid of claim 23, that is at least 85% identical to at least 50 consecutive nucleic acids SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; or SEQ ID NO: 19.

25. The nucleic acid of claim 24, that is at least 95% identical to at least 50 consecutive nucleic acids SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; or SEQ ID NO: 19.